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TITLE: Evaluation of Roles of Interferonγ Regulated Genes in Estradiol Inhibition of Androgen-Independent Prostate Cancer

PRINCIPAL INVESTIGATOR: Eva Corey, Ph.D.

CONTRACTING ORGANIZATION: University of Washington
Seattle, WA 98195-7720

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Eva Corey, Ph.D.

E-mail: ecorey@u.washington.edu

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14. ABSTRACT
Prostate cancer presents its greatest challenge to clinicians when it progresses to the hormone-independent state. Therapeutic methods which are effective regardless of androgen response, or even target androgen-independent CaP specifically, are of special medical and scientific interest. We have shown that estradiol (E2) can inhibit growth of hormone–independent prostate cancer in animal models. Among the genes up-regulated by E2 are interferon gamma (IFNγ)-regulated genes, which may be involved in *direct* growth inhibition by estrogen. The LuCaP 35V xenograft does not grow *in vitro*; for this reason, this exploratory proposal was design to evaluate the responses of various CaP cell lines to E2 and IFNγ *in vitro*.

The objectives of this proposal are to examine effects of E2 and IFNγ on CaP growth and apoptosis, and expression of IFNγ–regulated genes in hormone-independent CaP cells *in vitro*.

We have treated four CaP cell lines, DU145, PC-3, LNCaP, and C4-2B, with E2 and IFNγ, but to date none of the cell lines evaluated has exhibited properties similar to those of LuCaP 35V in vivo. DU145 cells were inhibited only by a high concentration of E2, while LNCaP and C4-2B were stimulated, and PC-3 were not affected. IFNγ did not affect proliferation of any of these cells. The expression changes in IFNγ–regulated genes subsequent to E2 treatment also did not resemble those detected in E2-treated LuCaP 35V in vivo. In the no-cost extension of this grant we will examine LAPC-4 cells, which express a wild-type androgen receptor.

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No subject terms provided.

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- Determination of effects on proliferation (0-6 months).
- Determination of effects on apoptosis (0-6 months).
- Determination of effects expression of immune-related genes by Real-time PCR, Flow cytometry, and Western blot (6-18 months).
- Examination of activation of STAT pathway after estradiol treatment (12-18 months).

Task 2. Examination of effects of Interferon gamma on CaP cells *in vitro*.

- Determination of effects on proliferation (0-6 months).
- Determination of effects on apoptosis (0-6 months).
- Determination of effects expression of immune-related genes by Real-time PCR and Western blot (6-18 months).
- Examination of activation of STAT pathway (12-18 months).

INTRODUCTION

Prostate cancer, the most common malignancy in American men, presents its greatest challenge to clinicians when it progresses to the hormone-independent state. Despite substantial attention, the development of androgen independence in CaP is not well understood, and current treatment methods are of limited value. Progression to the androgen-independent state represents in essence the loss of the primary signaling pathway used to control recurrent CaP following radical prostatectomy. Accordingly, therapeutic methods which are effective regardless of androgen response, or even target androgen-independent CaP specifically, are of special medical and scientific interest.

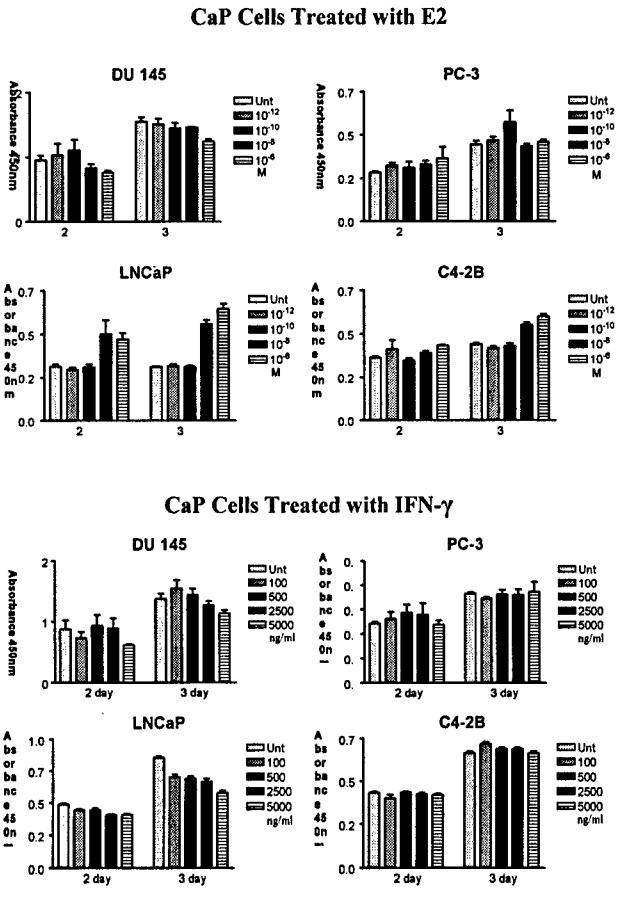
We have shown that estradiol (E2) can inhibit growth of hormone–independent prostate cancer in animal models. Expression of a variety of genes is upregulated by E2 treatment in the LuCaP 35V CaP xenograft. Among the genes up-regulated by E2 are interferon gamma (IFNγ)-regulated genes, which may be involved in *direct* growth inhibition by estrogen (as opposed to effects mediated by androgens or the immune system). The LuCaP 35V xenograft does not grow *in vitro*; for this reason, under this proposal we planned to evaluate the responses of various CaP cell lines to E2 and IFNγ *in vitro*. These studies should allow us to draw conclusions of two types: (1) what genes are consistently associated with growth inhibition of CaP by E2; and (2) the degree of congruence (in terms of gene expression) between the best *in vitro* model of this effect and the *in vivo* models we have described.

RATIONALE

The LuCaP 35V xenograft, which exhibits increased levels of IFN-regulated genes in response to E2 treatment, does not grow *in vitro*, and is therefore not amenable to detailed studies of signal transduction and phenotypic manipulations to determine the effects of altered gene expression. For this reason, under this exploratory proposal we evaluated the responses of various CaP cell lines to E2 and IFNγ *in vitro*. The studies were proposed to help establish whether increased expression of IFNγ and IFNγ-regulated genes associated with E2 treatment is an important aspect of the observed growth inhibition. Our results will help to determine the best *in vitro* model to use in a full hypothesis-driven proposal to examine the importance of the observed changes in inhibition of advanced prostate cancer.

RESULTS

Proliferation and Apoptosis:
Thus far we have examined effects of E2 and IFNγ on proliferation of four different prostate cancer cell lines. E2 slightly inhibited the proliferation DU 145 after 2 and 3 days of treatment, but only at high concentrations (10^{-6} and 10^{-8} M). PC-3 cells were unaffected. In contrast, E2 stimulated the growth of LNCaP and C4-2B, but this might be due to the mutated androgen receptor in these cells. IFN-γ treatment had no significant effects on proliferation of prostate cancer cells tested. Because no effects on cell number were seen, we did not evaluate apoptotic effects of E2 and IFN-γ on these cells.



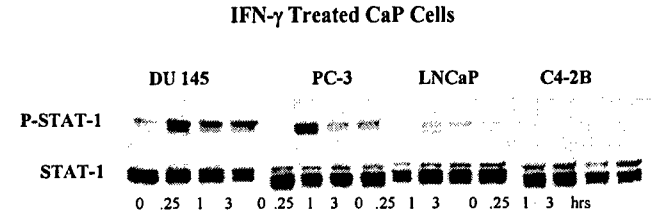
Expression of Immune-related Genes by Real-time PCR
We performed real-time PCR to examine the expression pattern of immune-related genes in four different prostate cancer cell lines and compared the results to changes in expression of these genes in E2-treated LuCaP 35V (Table 1.)

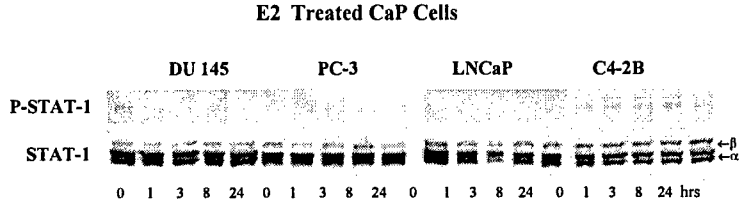
Table 1. Expression of IFN-regulated genes in CaP cells after E2 and IFNγ treatment. The results are presented as a fold change in comparison to untreated cells.

	IFN -treated					E2-treated			
normalized	DU 145	C4-2B	LNCaP	PC-3	LuCaP 35V	DU 145	C4-2B	LNCaP	PC-3
STAT-1	27.20	3.14	5.23	11.42	1.49	1.29	0.86	0.39	1.15
IFN 27	1.81	7.35	41.00	4.97	1.74	1.62	0.47	0.17	1.2
IFN 30	8.58	1.10	7.80	22.00	2.59	1.24	0.93	0.14	1.22
IFNP1	3.55	4.05	20.66	19.48	1.14	1.94	0.81	0.14	0.94
IFN TPR	19.05	5.88	26.34	3.78	2.32	2.37	0.17	0.24	0.55

Activation of JAK/STAT Pathway after E2 and IFN-γ Treatments

We observed activation of STAT-1 in LuCaP 35V treated with E2. Therefore we examined activation of STAT-1 in prostate cancer cells *in vitro* after treatment with E2 and IFN-γ. We observed that IFN-γ induced STAT-1 activation in DU-145, PC-3, and LNCaP, while E2 did not stimulate STAT-1 activation in any of the CaP cell lines tested.





KEY RESEARCH ACCOMPLISHMENTS

- Estradiol caused slight inhibition of proliferation of DU 145 prostate cancer cells at relatively high concentrations.
- Estradiol did not inhibit proliferation of PC-3 prostate cancer cells.
- Estradiol stimulated proliferation of LNCaP and C4-2B prostate cancer cells.
- INFγ did not inhibit proliferation of the prostate cancer cells tested.
- Treatment with E2 or INFγ did not result in alteration of IFN-regulated genes as E2 treatment of LuCaP 35V.
- E2 does not activate the STAT-1 pathway in prostate cancer cells *in vitro.*
- IFNγ activates the STAT-1 pathway in prostate cancer cells.

REPORTABLE OUTCOMES

None

CONCLUSIONS

Thus far we have not found an *in vitro* model capable of duplicating the E2 inhibition of LuCaP 35V *in vivo.* It is possible that inhibition by E2 is not activating IFN pathways directly. It is also possible that wild-type androgen receptor is required for these effects, and we have not tested prostate cancer cell lines with wild type androgen receptors. We have recently obtained LAPC-4 prostate cancer cells, which express wild-type androgen receptors (similarly to LuCaP 35V) and we will proceed to evaluate effects of E2 and IFNγ on LAPC-4 prostate cells *in vitro.*

However it is possible that the effects observed in LuCaP 35V *in vivo* may be an indirect effect through interactions of CaP cells with cells of the innate immune system or E2 activation of alternative pathways.